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Investigations of the Production, Transport, and Survival of Monoecious *Hydrilla* Propagules in the Tidal Potomac River

by Dwilette G. McFarland, John W. Barko

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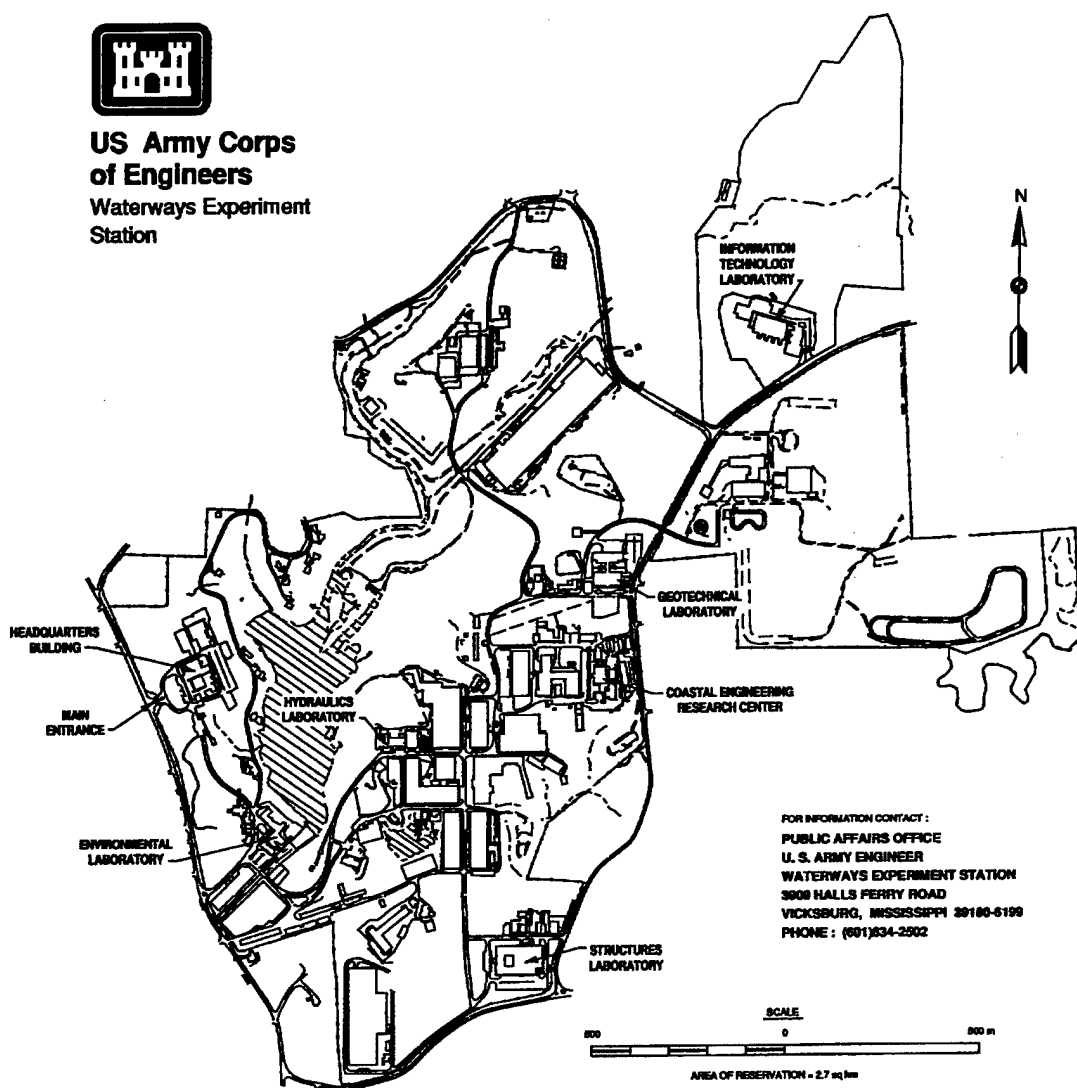
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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 33029. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Center for Aquatic Plant Research and Technology, Dr. John W. Barko, Director. Mr. Robert C. Gunkel was Assistant Manager for the APCRP. Program Monitor during these studies was Ms. Denise White, HQUSACE.

Principal Investigator was Ms. Dwilette G. McFarland, EL, WES. The report was prepared by Ms. McFarland and Dr. John W. Barko, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL. Experimental design, data analysis, and interpretation were provided by the authors with contributions from Dr. Craig Smith and Mr. Dennis Brandon, EPED, and from Ms. Nancy Rybicki, U.S. Geological Survey, Reston, VA. Technical assistance at EL was provided by Ms. Sue Fox and Mr. David Reed, ASCI Corporation, and contract students Ms. Maya Brown and Connie Austin and Messrs. Frank Boyd and Scott Fields, WES. Ms. Rybicki also coordinated sediment and propagule collection from Potomac River study sites. The report was reviewed within EL by Mr. R. Michael Stewart and Drs. John Madsen, Susan Sprecher, and Thomas Sturgis, EPED.

This research was performed under the general supervision of Dr. John W. Keeley, Director, EL, and Mr. Donald L. Robey, Chief, EPED, and under the direct supervision of Dr. Richard E. Price, Chief, EPEB.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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1 Introduction

Background

The submersed aquatic vegetation (SAV) of the freshwater tidal Potomac River has a history of dramatic change in abundance and distribution. Following a four-decade absence in the tidal river (between Chain Bridge and Quantico, VA; Figure 1), SAV reappeared in 1983 in the upper tidal reach and, by 1986, had spread farther downstream into the lower tidal reach as well (Paschal et al. 1982; Haramis and Carter 1983; Carter, Paschal, and Bartow 1985; Carter and Rybicki 1986, 1990; U.S. Geological Survey 1994, unpublished). Three submersed species, *Vallisneria americana* Michx., *Myriophyllum spicatum* L., and *Heteranthera dubia* (Jacq.) MacM., were dominant between 1983 and 1985; but by 1986, monoecious *Hydrilla verticillata* (L.f.) Caspary, a newly established exotic species (first documented near Dyke Marsh, Virginia, in 1982; Steward et al. 1984), had become most abundant. Since 1986, SAV in portions of the tidal river has declined, with the upper tidal reach experiencing the most severe losses (U.S. Geological Survey 1994, unpublished).

To better understand observed changes in the SAV communities, investigations of the tidal river were conducted to determine in situ factors affecting growth. Studies completed thus far have generally attributed changes in production and distribution patterns to changes in light availability and sediment and water quality characteristics (Carter, Paschal, and Bartow 1985; Carter and Rybicki 1985, 1990). However, other factors influencing propagule abundance and viability may also be involved. Information on in situ conditions potentially affecting propagule production and early stages of growth would be useful in predicting the future survival and distribution of SAV in the river.

In March 1994, the Environmental Laboratory (EL) of the U.S. Army Engineer Waterways Experiment Station (WES) and the U.S. Geological Survey began joint investigations of the production, transport, and survival of propagules of submersed aquatic macrophytes in the tidal Potomac River. The studies conducted at WES (reported here) focused on monoecious *Hydrilla verticillata* (L.f.) Caspary, a prolific species that produces a variety of propagule types (i.e., turions, fragments, rhizomes, stolons, and seeds).

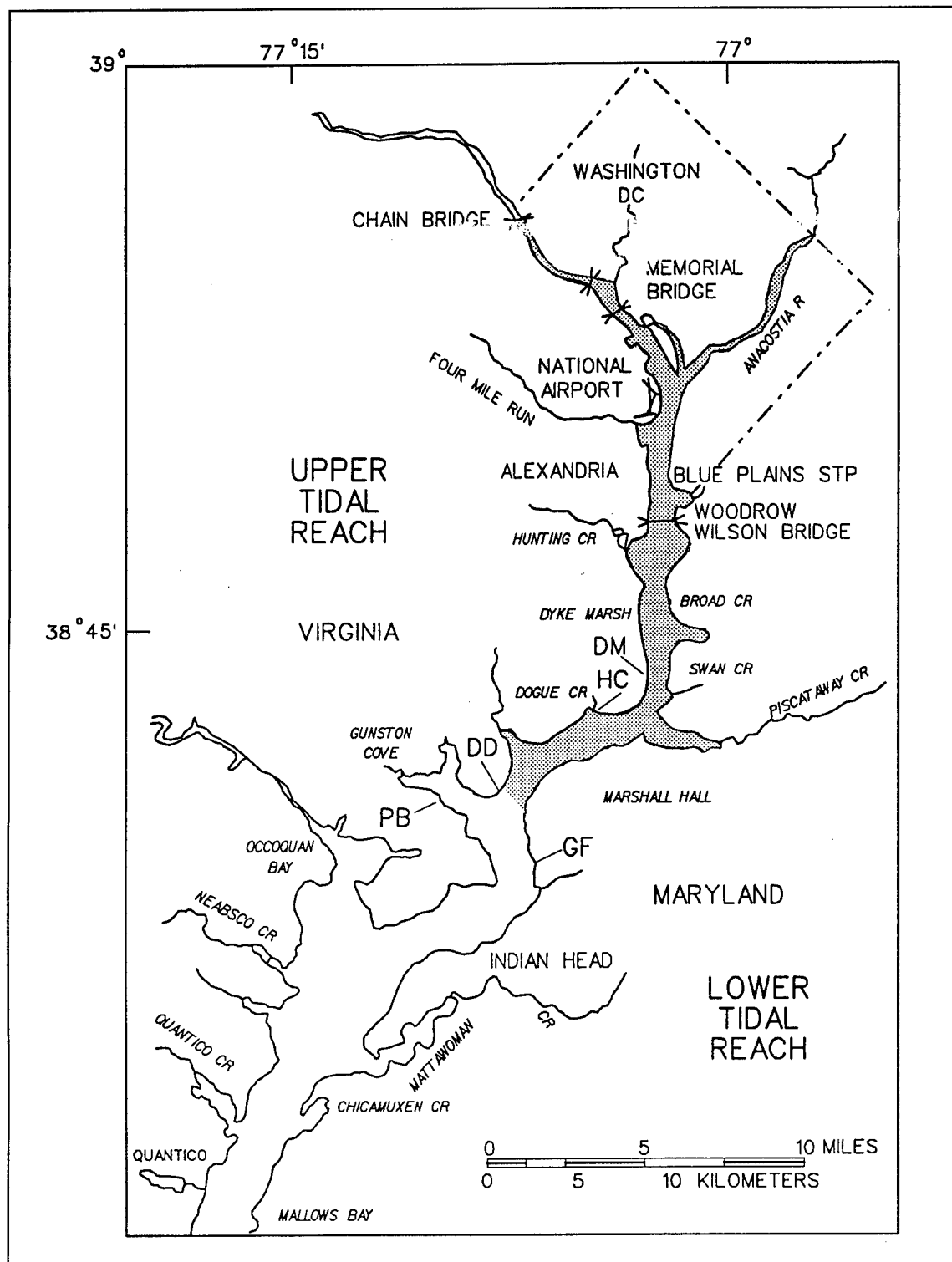


Figure 1. Location of study sites in Potomac River, 1994

These studies were specifically designed to assess viability of subterranean and axillary turions and to examine growth of fragments exposed to river conditions for different periods of time. Also, to enhance understanding of factors that may limit propagule growth in the river, growth of *Hydrilla* fragments was evaluated on sediments from different Potomac River sites.

Perennation and Spread of Monoecious *Hydrilla*

In the tidal Potomac River, *Hydrilla* overwinters in the form of subterranean and axillary turions that germinate in spring as temperatures rise (Carter, Rybicki, and Schulman 1987). Production of these propagules occurs mainly in the fall (under photoperiods < 12 hr; Steward and Van 1984; Spencer and Anderson 1986), although some tuberization has been reported in the river in late June and July (photoperiod \approx 14 hr; Carter, Rybicki, and Schulman 1987; Van 1989). Axillary turions (also called green winter buds) develop in the axils of leaves on stems above ground (Sculthorpe 1967; Pieterse 1981). When fully formed, they may fall directly onto the sediment or can be carried up and down stream by water currents and tides. Subterranean turions (also referred to as tubers or brown turions) are anatomically similar to axillary turions but are formed below ground at the ends of positively geotropic shoots (Sculthorpe 1967; Pieterse 1981). Since they are buried in sediment, subterranean turions are less likely than axillary turions to move from place to place; however, continued wave action and currents causing sediment scour can potentially uncover these propagules and sweep them away from their original site (Carter, Rybicki, and Schulman 1987).

During a single growing season, lateral expansion of *Hydrilla* can occur rapidly from production of rhizomes or runners that root at the nodes and form new shoots (Yeo, Falk, and Thurston 1984). Field samples of populations in the Potomac River have shown evidence of perennating rhizomes (K. K. Steward, as cited in EL 1985), but these structures are thought to be of minor importance relative to other modes of perennation, e.g., tubers and turions (Van and Steward 1990 and literature therein).

The colonization of new and distant habitats by *Hydrilla* is made possible through fragmentation. The long delicate stems of this species are easily broken, particularly during senescence, and can be easily dispersed over long distances by wave action and strong currents (Haller 1976; Anderson and Dechoretz 1982). After an initial period of flotation, the fragments eventually lose buoyancy and settle onto bottom sediments where they may become rooted and continue to grow (Steward 1992). Theoretically, any fragment of a stem that contains at least one node and intact leaves is capable of producing a new plant (Pieterse 1981); however, experimental evidence shows that fragments having two or more nodes have a higher frequency of new shoot production (Langeland and Sutton 1980; Anderson 1984).

In *Hydrilla*, as in many submersed macrophyte species, vegetative (asexual) reproduction is generally more important than sexual reproduction

for perennation and dispersal (Sculthorpe 1967; Pieterse 1981; Haynes 1988). Although monoecious *Hydrilla* can produce viable seeds (Conant, Van, and Steward 1984; Langeland and Smith 1984; Steward 1993), there is a notable lack of information concerning seedling survival, particularly in situ. Recently, monoecious *Hydrilla* was found to produce seeds rather infrequently in comparison with other species that seed annually in the Potomac River.¹ Floral biology and pollination in monoecious plants have been described in detail by Yeo, Falk, and Thurston (1984) and Cook and Luond (1982) and reviewed by Pieterse (1981). Flowering in this biotype is apparently induced under short days (Pieterse 1981). Although ample pollen is released by male flowers, pollination of female flowers is often unsuccessful (Cook and Luond 1982). Considering the lack of observations of seedling survival and the apparent inefficiency of pollination in nature, it is unlikely that seed production significantly affects the abundance of *Hydrilla* in the tidal river.

Study Sites in Potomac River

Five sampling sites were established in the freshwater tidal Potomac River near Washington, DC (Figure 1). According to recent U.S. Geological Survey data (for 1993; see Table 1), two of the five sites were vegetated with 40- to 70-percent areal coverage in SAV: (a) Greenway Flats (GF) was covered predominantly with *Myriophyllum* and *Hydrilla*; and (b) the Dempsey Dumpster (DD) site, mainly with *Vallisneria* and *Myriophyllum*. The three remaining sites were essentially unvegetated: (a) Hunting Creek (HC) had no SAV; (b) DM-4R (DM), only trace amounts of *Potamogeton pectinatus* L. and *Hydrilla*; and (c) Pohick Bay (PB), no SAV. Notably, in 1988, the HC and DM sites each had 100-percent areal coverage by *Hydrilla*; however, the PB site has never supported SAV (U.S. Geological Survey 1994, unpublished).¹

¹ Personal Communication, 1995, Nancy Rybicki, Hydrologist, U.S. Geological Survey, Reston, VA.

Table 1
Percent Areal Coverage and Species Composition of Submersed
Macrophyte Communities in Tidal Potomac River (derived from
Rybicki and Carter 1995)

Site	1988		1993	
	Areal Coverage, %	Dominant Species, % ¹	Areal Coverage, %	Dominant Species, %
Vegetated				
GF	100	Hyd: 100	40 - 70	Myr: 80 Hyd: 20
DD	40 - 70	Hyd: 60 Val: 30 Myr: 5 Het: 5	40 - 70	Val: 80 Myr: 20
Unvegetated				
HC	100	Hyd: 100	0	n.a.
DM	100	Hyd: 100	<10	Hyd: trace Pot: trace
PB	0	n.a.	0	n.a.
¹ Species abbreviations: Het = <i>Heteranthera dubia</i> ; Hyd = <i>Hydrilla verticillata</i> ; Myr = <i>Myriophyllum spicatum</i> ; Val = <i>Vallisneria americana</i> ; Pot = <i>Potamogeton pectinatus</i> ; n.a. = not applicable.				

2 Methods and Materials

Study 1: Germination of Tubers and Turions

In March and again in December (i.e., before and after the growing season) of 1994, all five sites were sampled by the U.S. Geological Survey for *Hydrilla* tubers and turions. At each site, sampling was conducted at four to five stations along a transect perpendicular to the shoreline; transects were generally terminated at a depth of 2.0 m at low tide. Twenty core samples were taken with a posthole digger (308 cm²) at each station with a water depth ≤ 1.5 m; at deeper stations, 10 grabs (changed to 5 grabs in December sampling) were taken with modified oyster tongs (900 cm²). Cores obtained with the posthole digger ranged from 10 to 20 cm in depth depending on sediment texture. Oyster tongs usually penetrated sediment to a depth of ≈ 10 cm.

Tubers and turions in the sampled sediments were removed by sieving through a fine-mesh screen. The collected propagules were refrigerated (at 6 °C) and shipped to WES in coolers within 5 days; there they were transferred to 6-l battery jars of low alkalinity culture solution (after Smart and Barko 1985) and held in cold storage (at 6 to 8 °C) for approximately 2 1/2 weeks.

Germination of the propagules was observed under greenhouse conditions in the EL, WES. Large (1,200-l) white fiberglass tanks were filled approximately 50 cm deep with the same culture solution as indicated above. The solution was maintained at 25 (± 1) °C and aerated with humidified air to enhance mixing and air/water CO₂ exchange. Maximum midday photosynthetically active radiation inside the tanks reached moderate levels (300 to 350 $\mu\text{E m}^{-2} \text{s}^{-1}$) using neutral density shade fabric over the greenhouse roof.

Tubers and turions of *Hydrilla* were planted in sediment collected from Brown's Lake, WES. The sediment was thoroughly mixed and poured to a depth of 7 cm in 300-ml plastic cups. Tables 2 and 3 summarize fundamental sediment characteristics determined using analytical procedures described below (see methods for Study 2). Prew weighed propagules were planted singly per cup and observed daily for sprouting over a period of 2 weeks. Fresh weights determined just prior to planting were later used in assessing the viability of propagules in different size groups.

Table 2
Sediment Physical Characteristics¹

Source	Particle Size, %		Organic Matter %	Moisture Content %	Density mg/l
	Coarse > 50 μ diam	Fine \leq 50 μ diam			
Brown's Lake	9.2 c	90.8 a	4.24 a	39.5 a	0.88 b
Pohick Bay	86.7 a	13.3 c	1.81 c	27.3 b	1.23 a
DM-4R	86.7 a	13.3 c	2.20 b	27.7 b	1.17 a
Hunting Creek	67.5 b	32.5 b	2.06 bc	29.0 b	1.17 a

¹ Values are means based on analysis of three replicate sediment samples. Within each column, means sharing the same letter do not differ at the 5-percent level of significance (Duncan's Multiple Range Test).

Table 3
Sediment Nutrient Concentrations¹

Source	Exchangeable Nitrogen, NH ₄ -N mg/g	Available Phosphorus, PO ₄ -P mg/g
Brown's Lake	0.069 \pm 0.001 a	0.157 \pm 0.014 a
Pohick Bay	0.034 \pm 0.001 b	0.068 \pm 0.002 c
DM-4R	0.019 \pm 0.001 c	0.110 \pm 0.001 b
Hunting Creek	0.017 \pm 0.000 d	0.065 \pm 0.001 c

¹ Values are means \pm standard errors based on analysis of three replicate sediment samples. Within each column, concentrations sharing the same letter do not differ at the 5-percent level of significance (Duncan Multiple Range Test).

Study 2: Sediment Bioassay

The investigation was conducted in April 1994 in a controlled growth chamber at the EL, WES. This facility was operated to maintain 25 °C and a 14-hr photoperiod at 350 μ E m⁻² s⁻¹. *Hydrilla* fragments were grown in 20-l lucite columns (150 cm tall) with removable 3.5-l bases (Figure 2). A detailed description of the environmental chamber and ancillary equipment is presented in Barko and Smart (1980).

Sediments used in the study were collected in March from Brown's Lake, WES (reference sediment), and from the three unvegetated Potomac River sites: DM, PB, and HC (Figure 1). Each sediment was mixed separately and placed into 1-l plastic containers with a surface area of 90.03 cm². Six

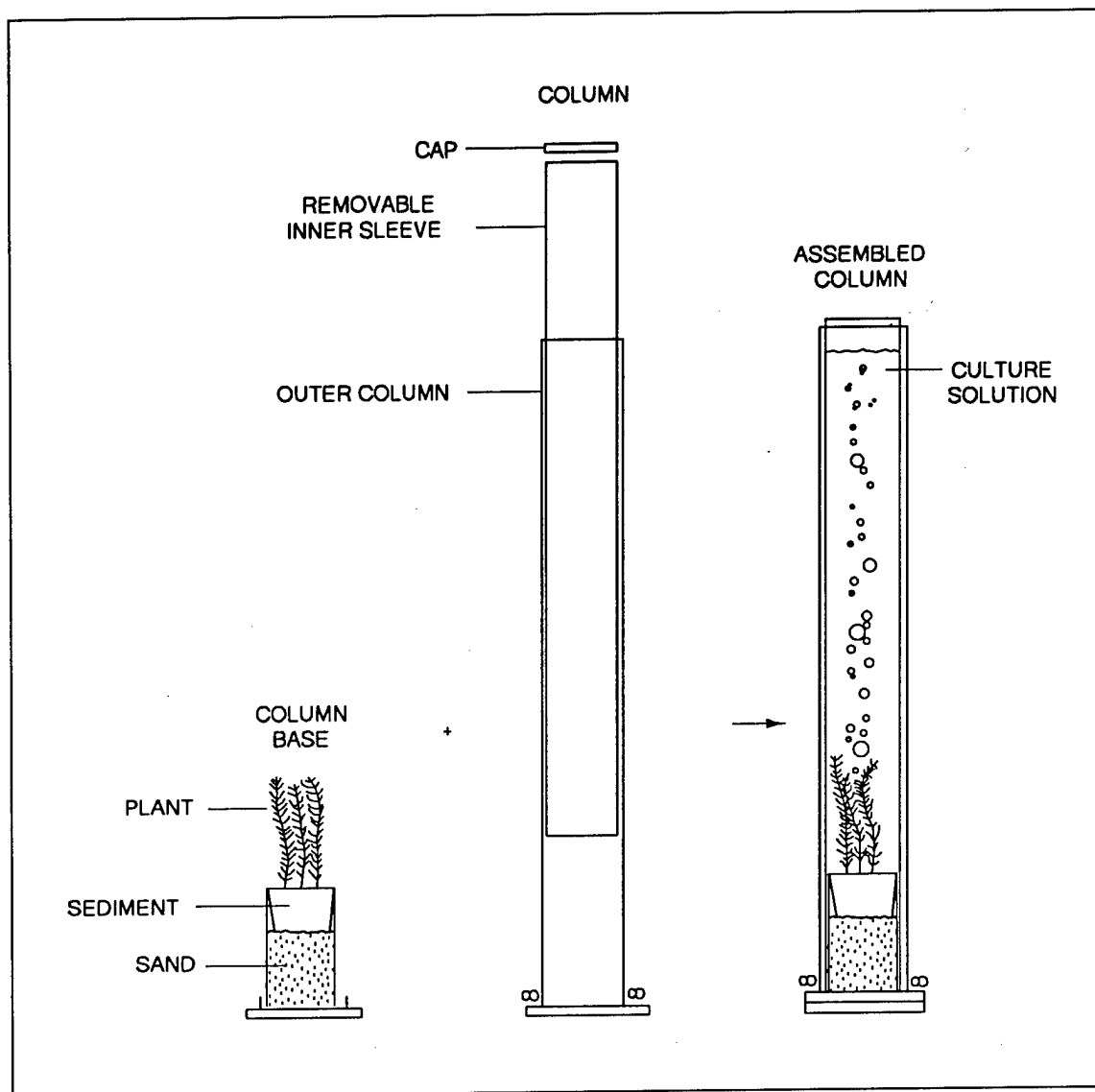


Figure 2. Assembly of lucite columns used in Potomac River sediment bioassay

replicates per sediment were allowed to settle and equilibrate prior to planting using procedures in Barko and Smart (1986); afterwards, they were transferred individually into column bases filled with 2 l of washed silica sand (Figure 2).

Initial sediment samples were analyzed for physical and chemical characteristics. Particle-size distribution (texture) was determined using the hydrometer method of Patrick (1958). Sediment moisture content and density were evaluated gravimetrically after oven-drying measured volumes of sediment at 105 °C. Dried samples were placed in a muffle furnace and combusted at 550 °C for estimations of organic matter content from loss of mass following ignition (Allen et al. 1974). Exchangeable ammonium-N was obtained by extraction with 1 M NaCl in a cation exchange procedure modified from

Bremner (1965). Available P was obtained using 0.030 N NH_4F and 0.025 N HCl (after Olsen and Sommers 1982). N and P concentrations were determined colorimetrically with a Technicon Autoanalyzer II, employing a molybdate method for P and a salicylate method for N (American Public Health Association (APHA) 1989).

Apical cuttings of *Hydrilla*, 20 cm in length, were obtained from a 6-week-old EL culture stock. This stock was originally established from a collection made earlier near the PB site (Figure 1). Three sprigs of *Hydrilla* were planted per experimental column and submerged in 15 ℓ of culture solution prepared in the same manner as in the previous study. Filtered humidified air was delivered to the columns through plastic air dispersers positioned at the base of the plants.

After 5 weeks of growth, aboveground and belowground plant structures were harvested, oven-dried at 80 °C, and weighed. Total biomass (as the sum of aboveground and belowground biomass), root-to-shoot ratio, and plant height were used as determinants of growth. Dried shoot tissues were finely ground to a particle size < 0.7 mm and digested with sulfuric acid and hydrogen peroxide according to Allen et al. (1974). N and P concentrations in the resulting digestates were subsequently determined using autoanalyzer procedures described for sediment samples above (APHA 1989). The accuracy of analytical procedures used to obtain tissue nutrient concentrations was checked by including reference tissues of the National Bureau of Standards in all experimental sample sets.

Study 3: Fragment Viability Over Time

In July 1994, apical cuttings (i.e., allofragments) of *Hydrilla* were obtained from a healthy population located \approx 3 km downstream from the PB site (Figure 1). The fragments were adjusted to a length of 15 cm and divided into four groups: one group was used for initial (Week 0) determinations (see below), and the three remaining groups were allowed to float in the river at the DM (unvegetated) site for respective periods of 1, 2, and 4 weeks. Fragments held under river conditions were placed inside exclosures constructed using cylindrical cages (60 cm long by 15 cm diam) covered with 0.5-cm mesh and securing buoys for flotation (see Rybicki and Carter (1995) for details).

At the EL, subsamples from each exposure group (i.e., 0, 1, 2, and 4 weeks) were used to assess fragment biomass (oven-dried at 80 °C), morphological characteristics (i.e., length of main stem and number of apices), and nutritional status (i.e., total N and P, and internal carbohydrate content) prior to viability testing. Concentrations of N and P in fragment tissues were determined following digestion in a sulfuric acid/hydrogen peroxide mixture (Allen et al. 1974), using procedures described earlier for automated tissue analysis (APHA 1989). Internal carbohydrate (i.e., free sugar, starch, and total nonstructural carbohydrate) content was determined and expressed on a

per fragment basis after Nelson (1944), Swank et al. (1982), and Luu and Getsinger (1990).

Viability of the fragments was tested under greenhouse conditions similar to those described for Study 1. Twenty fragments per exposure were planted individually in small (300-ml) plastic cups. Each cup was filled to a depth of 7 cm with well-mixed sediment collected from Brown's Lake, WES (characterized in Tables 2 and 3). A thin layer of washed silica sand (< 1 cm deep) was used as a sediment cover. The fragments were planted with basal ends buried \approx 5 cm below the sand surface and, for accuracy, were checked for height and number of apices (above ground) just prior to submersion.

After 3 1/2 weeks in culture, the plants were clipped at the sand surface and examined immediately for shoot height and branching. Belowground biomass was retrieved by rinsing with water through a 1-mm mesh sieve. Total biomass was assessed from separate determinations of aboveground and belowground (oven-dried) plant mass. Within each exposure group, fragments showing significant increases in biomass, shoot height, and/or number of apices were considered to be viable. Since the change in dry mass per fragment could not be determined directly from the same individual, estimates were obtained indirectly by subtracting the average biomass per individual before viability testing ($n = 20$ fragments per exposure) from the actual final biomass per fragment 3 1/2 weeks after planting.

For comparison with laboratory results, eight sets of 10 fragments from each exposure group (0, 1, 2, and 4 weeks) were placed in flats on the river bottom at the DM site. Each flat consisted of a 0.5- by 0.5-m mesh bag held in shape by 20- by 60-cm wire fencing (described further in Rybicki and Carter 1995). Four sets per exposure group were placed at a depth of 0.5 m and another four sets at a depth of 0.8 m. Two sets of fragments per depth/exposure combination were observed for rooting over 2- and 4-week periods.

Study 4: Number and Viability of Transported Propagules

From May to September, two types of collection devices were deployed to determine whether fragments of submersed aquatic macrophytes were being transported to the unvegetated study sites (DM, HC, and PB; Figure 1). Nets were used to collect fragments floating on the surface and suspended within the upper 0.6 m of the water column, and traps were placed on the sediment to catch fragments carried along and settling on the river bottom. Three nets and 12 traps were positioned at each of the three sites. For further details on the construction and deployment of these apparatuses, and regarding fragment collection, see Rybicki and Carter 1995.

At WES, fragments of *Hydrilla* were counted from each trap and net, and fresh weight per apparatus was determined. Depending on the number of fragments in each sample, viability testing (similar to that described in Study 3) was performed either on the total number of fragments per apparatus or on a substantial portion (usually between 25 and 50 percent) of that number.

Data Analysis

Data obtained here were analyzed using analysis of variance (ANOVA) and post-ANOVA procedures of the Statistical Analysis System (SAS Institute, Inc. 1991). Comparisons of means were accomplished using Duncan's Multiple Range Test and Dunnett's Procedure. Hereafter, statements of statistical significance refer to probability levels of 5 percent or less.

3 Results and Specific Discussion

Study 1: Germination of Tubers and Turions

Preseason propagule assessments

Nearly all overwintered tubers and turions of *Hydrilla* were found at the GF (vegetated) site (Table 4). The general absence of these propagules at the other sampling sites was not particularly surprising since, during the previous growing season, three of the five sites (PB, HC, and DM) had been unvegetated and the remaining vegetated site (DD) had been occupied mostly by *Vallisneria*.

Table 4
Percentage Germination of *Hydrilla* Propagules Collected in March 1994 (pre-growing season) From Different Sampling Sites in Tidal Potomac River

Site	Tubers			Turions			Propagules ¹		
	Total (= n)	No. Germ.	% Germ.	Total (= n)	No. Germ.	% Germ.	Total (= n)	No. Germ.	% Germ.
Vegetated									
GF	49	38	78	81	49	60	130	87	67
DD	0	0	n.d. ²	1	0	0	1	0	0
Unvegetated									
PB	0	0	n.d.	0	0	n.d.	0	0	n.d.
DM	0	0	n.d.	0	0	n.d.	0	0	n.d.
HC	0	0	n.d.	0	0	n.d.	0	0	n.d.
All Sites							131	87	66

¹ Propagules = tubers + turions.
² n.d. = no data.

The percentage germination of tubers of *Hydrilla* harvested from different depths at the GF site is listed in Table 5. No tubers were found at the shallowest (0.5-m) sampling depth (Station 1). Tubers collected from water depths of 1.0 to 1.1 m (Stations 2 and 3) occurred in the greatest densities and, under laboratory conditions, exhibited the highest germination percentages. Less than half of the tubers harvested from the 1.5-m sampling depth (Station 4) and none of those from the 2.0-m depth (Station 5) germinated. For all stations at the GF site combined, tuber germination ($n = 49$) was approximately 78 percent (Table 4).

Table 5
Percentage Germination of Tubers and Turions of *Hydrilla* Collected in March 1994 (pregrowing season) From Different Sampling Stations at Greenway Flats (GF) Site, Potomac River

Station	Depth, m	Density no./sq m ¹	Number Collected, n	Percentage Germination ²
Tubers				
1	0.5	0.0	0	n.d.
2	1.0	49.5	18	100
3	1.1	65.9	24	75
4	1.5	13.7	5	40
5	2.0	2.2	2	0
Turions				
1	0.5	2.7	1	100
2	1.0	0.0	0	n.d.
3	1.1	0.0	0	n.d.
4	1.5	156.6	57	54
5	2.0	24.8	23	74
¹ Density calculations are based on the number of propagules collected using sampling techniques described in the Methods section of this report. ² n.d. = no data.				

Tubers of *Hydrilla* ranged in fresh weight from 13 to 306 mg. Examination of tuber frequency in different size (fresh weight) categories revealed a distribution pattern that was slightly skewed to the left (Figure 3). Tubers in the smallest size category (< 81 mg) accounted for approximately 30 percent of the total population, but the percentage germination in these propagules was quite low (50 percent). Although relatively few tubers, accounting for only 12 percent of the population, occurred in the largest size category (241 to 320 mg), the percentage germination was highest (100 percent) in this group.

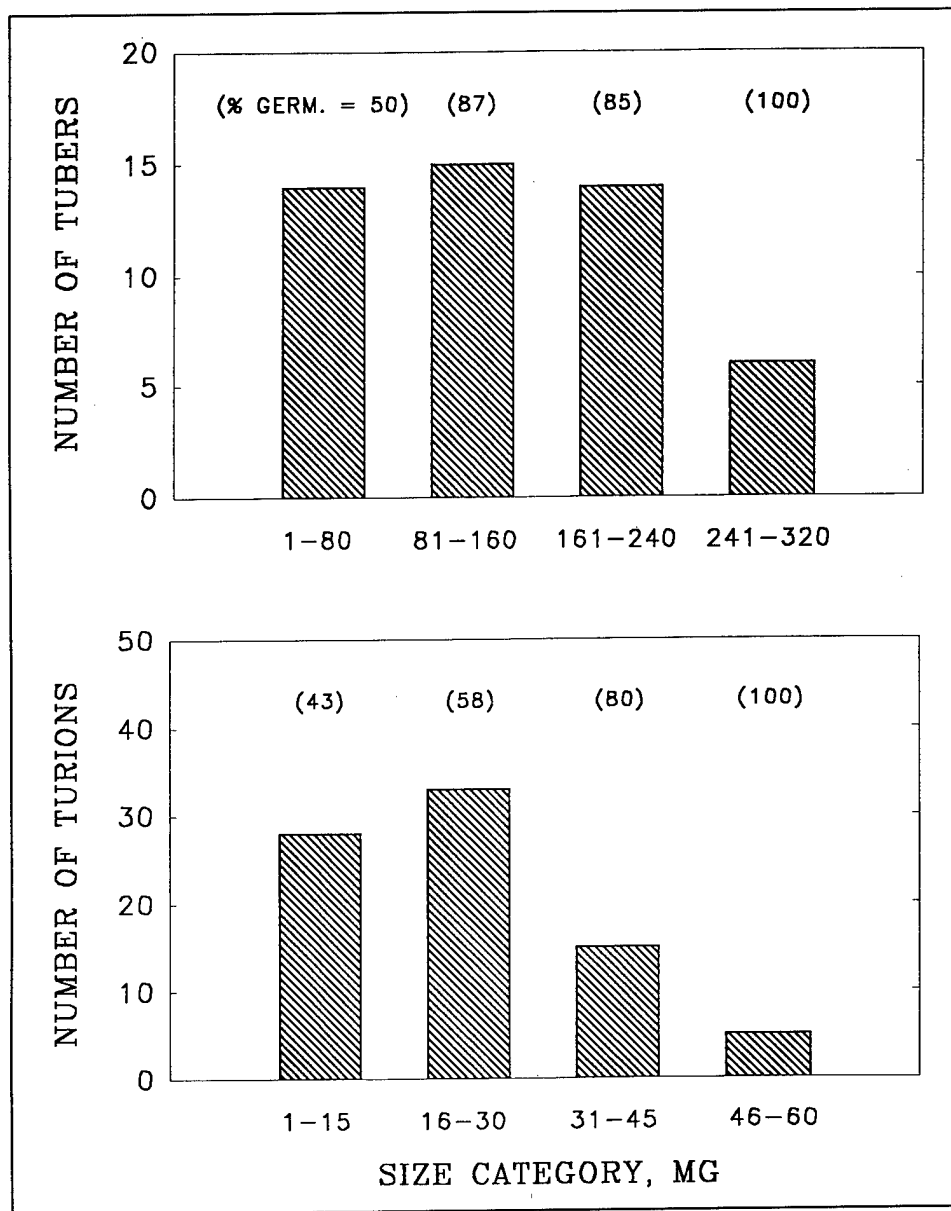


Figure 3. Number of tubers (top frame) and turions (bottom frame) of *Hydrilla* in different size categories by fresh weight collected from Greenway Flats (GF) site (Percentage germination within each size category is indicated in parentheses)

Eighty-one turions of *Hydrilla* were collected from the GF site—most of which were obtained from sampling depths of 1.5 to 2.0 m (Stations 4 and 5; Table 5). On the whole, only 60 percent of these propagules germinated successfully under favorable growth conditions, perhaps because many in this population were extremely small. Turions ranged in fresh weight from 4 to 60 mg and, similar to the tuber population, exhibited increased germination with increasing propagule size (Figure 3).

Based on pre-season propagule data, mean fresh weight per tuber (146 mg; $n = 49$) was greater than the mean fresh weight per turion (24 mg; $n = 82$, including the one found at site DD). The relatively smaller size of turions has been observed in numerous populations of *Hydrilla* (Spencer et al. 1987; McFarland and Barko 1993), despite anatomical similarities between these two propagule types (Pieterse 1981; Yeo, Falk, and Thurston 1984). Spencer et al. (1987) have proposed that the difference between tuber and turion size may result from different survival strategies. Turions form above ground and thus are more likely to be dispersed by water movements to colonize new habitats. Selective pressures on these propagules would favor, to some extent, a smaller size to aid in dispersal. In contrast, tubers form below ground and, unless there is significant sediment disturbance, are less likely than turions to be transported to other locations.

Postseason propagule assessments

Collections in December 1994 revealed that tubers and turions of *Hydrilla* were absent from the HC and PB sites, and those occurring at sites GF, DM, and DD were exceedingly scarce (see Table 6 for totals). At GF, 1 tuber and 2 turions were obtained from the two shallowest stations, resulting in estimated densities of 2.7 tubers and 5.5 turions/m², at respective 0.5-m and 1.0-m sampling depths. Two turions of *Hydrilla* were also found at the DM site, with an estimated density of 5.5 turions/m² at the 0.5-m sampling depth. Only one propagule of *Hydrilla* (a turion) was obtained at the DD site, and it was collected from the deepest sampling station. Thus, the estimated density at the 2.0-m sampling depth was ≈ 2.1 turions/m².

Table 6 Percentage Germination of <i>Hydrilla</i> Propagules Collected in December 1994 (postgrowing season) From Different Sampling Sites in Tidal Potomac River									
Site	Tubers			Turions			Propagules ¹		
	Total (= n)	No. Germ.	% Germ.	Total (= n)	No. Germ.	% Germ.	Total (= n)	No. Germ.	% Germ.
Vegetated									
GF	1	0	0	2	2	100	3	2	67
DD	0	0	n.d. ²	1	1	100	1	1	100
Unvegetated									
PB	0	0	n.d.	0	0	n.d.	0	0	n.d.
DM	0	0	n.d.	2	1	50	2	1	50
HC	0	0	n.d.	0	0	n.d.	0	0	n.d.
All Sites							6	4	67
¹ Propagules = tubers + turions. ² n.d. = no data.									

Table 6 provides germination percentages for tubers and turions of *Hydrilla* collected during postseason field samplings. Of the six propagules that were obtained, two did not germinate: one tuber (fresh weight = 4 mg) from the GF site and one turion (fresh weight = 14 mg) from the DM site. Germination among turions ($n = 5$; fresh weight from 14 to 36 mg) was 80 percent. However, in calculations including the tuber (1) from the GF site, germination fell overall to only 67 percent. The scarcity of healthy propagules to overwinter at these sites suggested that, at the onset of the next growing season, the population of *Hydrilla* (if present in those areas) would probably be very sparse.

Study 2: Sediment Bioassay

Determinations of sediment compositional characteristics (Table 1) revealed that Brown's Lake (i.e., BR; reference) sediment was finer in texture than sediments from the unvegetated Potomac River sites (i.e., HC, PB, and DM). Due to higher sand content (i.e., coarse particle fraction), PB, HC, and DM sediments each had greater bulk densities and less moisture than did sediment from BR. Organic matter content was greatest for BR sediment, but in all cases fell within the lower range reported for many North American lakes (Barko and Smart 1986).

Initial concentrations of extractable nutrients differed considerably among the studied sediments, with BR having the highest concentrations of exchangeable N and available P (Table 2). Among sediments from the unvegetated sites, exchangeable N levels were highest in PB sediment, while available P levels were highest in the sediment from DM. HC sediment had the lowest concentrations of exchangeable N and, along with PB sediment, the lowest concentrations of available P.

By the end of the 5-week study period, plants on BR sediment were clearly taller than those on any of the three Potomac River sediments and had significantly greater biomass than those on sediments from sites DM and HC (Figure 4). Growth was least on site HC sediment where total biomass was 25 percent lower and plant height 40 percent lower than on sediment from BR. Biomass production was similar on BR and PB sediment. Results of correlation analyses showed that plant height and biomass responses were significantly related to levels of exchangeable N ($r > 0.8$, $P < 0.01$, in both cases) but not available P. Root-to-shoot ratios (though somewhat higher on HC and DM sediments) showed no significant sediment effects.

Table 7 lists concentrations of N and P in shoot tissues of *Hydrilla* planted on sediment from BR and three Potomac River sites. Critical (i.e., growth-limiting) concentrations of N and P have not been determined specifically for monoecious *Hydrilla*, but for the dioecious biotype, these values are estimated to be $9.2 (\pm 0.4)$ mg N (Smart, Barko, and McFarland 1994) and 0.7 mg P

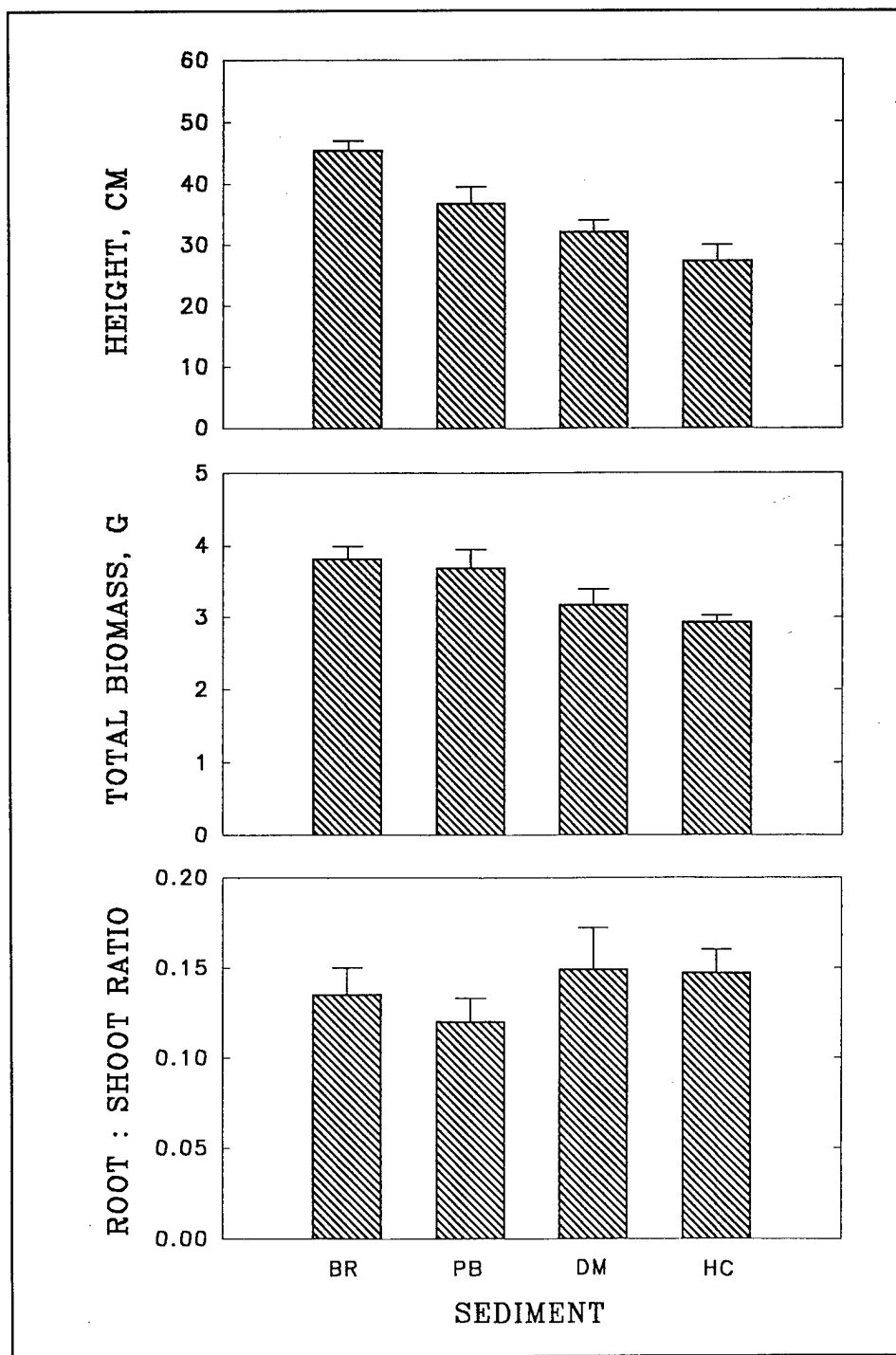


Figure 4. Plant height (top frame), total biomass (middle frame), and root-to-shoot ratios (bottom frame) of *Hydrilla* grown on a reference sediment from Brown's Lake (BR), WES, and on sediments from three unvegetated sites in the Potomac River: Pohick Bay (PB), DM-4R (DM), and Hunting Creek (HC) (Values are means with standard errors based on six replicate determinations)

Table 7
Nutrient Concentrations in Aboveground Tissues of *Hydrilla*
Planted on Sediments From Brown's Lake, WES, and Potomac
River (unvegetated) Sites¹

Source	Dry Tissue	
	Nitrogen, mg/g	Phosphorus, mg/g
Brown's Lake	16.51 ± 1.44 a	3.94 ± 0.15 a
Pohick Bay	15.87 ± 1.58 a	3.76 ± 0.28 a
DM-4R	10.89 ± 0.82 b	2.72 ± 0.20 b
Hunting Creek	9.18 ± 0.45 b	2.98 ± 0.17 b

¹ Values are means ± standard errors based on analysis of six replicate tissue samples. Within each column, concentrations sharing the same letter do not differ at the 5-percent level of significance (Duncan Multiple Range Test).

per gram dry plant tissue (Steward 1972). Also, concentrations of 13 mg N and 1.3 mg P per gram are generally considered to be critical for the growth of other submersed aquatic macrophytes (Gerloff and Krombholz 1966). Based on the above values, N concentrations reached critically low levels in *Hydrilla* planted on HC and DM sediments, but were apparently sufficient for growth on sediments from BR and PB. P concentrations in *Hydrilla* tissues were in all instances well above critical levels.

Study 3: Fragment Viability Over Time

Apical cuttings of *Hydrilla* (i.e., allofragments) left floating in the river experienced marked changes in biomass and morphology (Figure 5). After 1 week of flotation, the fragments showed significant increases in biomass and number of apices, but were about the same length ($P > 0.05$) as when originally cut. Initial root development and branch elongation were evident in fragments that had floated for 2 weeks; however, these fragments were somewhat shorter along their main stems ($P < 0.05$) due to breakage and had less biomass ($P < 0.05$) than those in the previous (1-week) exposure group. The condition of fragments after 4 weeks of flotation was generally very poor; root structures and lower leaves had been lost apparently to grazers (Chironomidae and *Gammarus* spp. were observed), and there were signs of further breakage, yellowing, and some leaf/stem deterioration.

Tissue N concentrations in the fragments were relatively high during the first week (Table 8) but declined markedly thereafter (by ≈ 85 percent) to levels indicative of N-deficiency stress (see critical concentrations proposed by Gerloff and Krombholz (1966) and Smart, Barko, and McFarland (1994) as discussed above). Observed elongation of fragment roots and apices (branches) may have contributed to the dilution of N in the tissues. P concentrations varied to a minor extent ($P = 0.0117$, F value = 7.19) compared

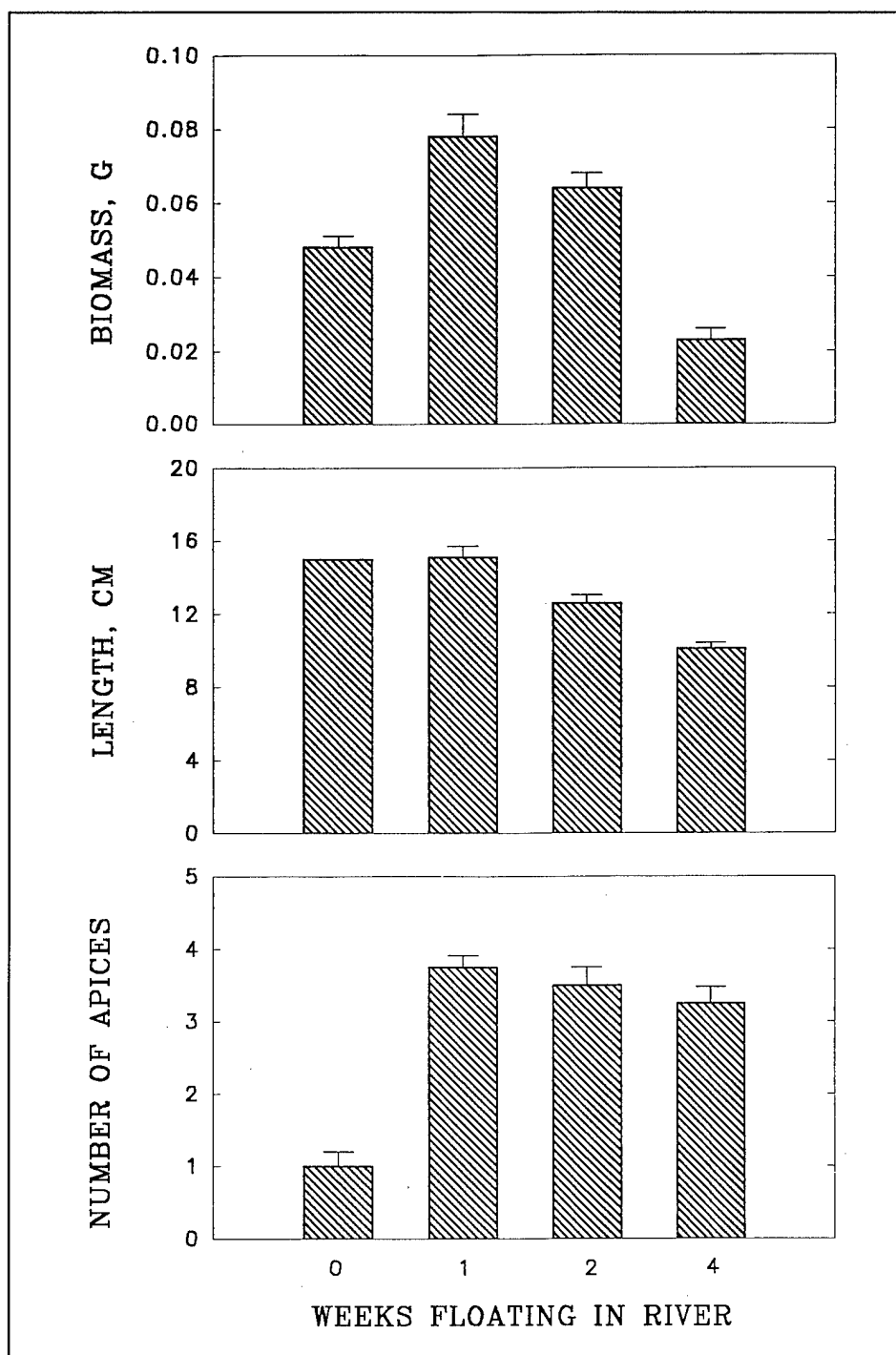


Figure 5. Biomass (top frame), length (middle frame), and number of apices (bottom frame) of *Hydrilla* fragments immediately after floating for different time intervals in tidal Potomac River, DM-4R site (Values are means with standard errors based on 20 replicate determinations)

Table 8

Variations in Total N and P Concentration and Content (i.e., product of nutrient concentration and biomass) in *Hydrilla* Fragments Over Time¹

Weeks Floated in River	Concentration		Content	
	mg N g ⁻¹	mg P g ⁻¹	mg N frag ⁻¹	mg P frag ⁻¹
0	25.3 b	3.03 b	0.95 b	0.11 b
1	27.8 a	3.26 b	2.45 a	0.28 a
2	3.8 c	5.12 a	0.22 c	0.30 a
4	3.5 c	2.70 b	0.08 d	0.06 b

¹ Values are means based on analysis of three replicate dry tissue samples. Within each column, means sharing the same letter do not differ at the 5-percent level of significance (Duncan's Multiple Range Test).

with tissue N ($P = 0.0001$, F value = 1794.78) and in all exposures were maintained above suggested critical levels (cf. Gerloff and Kromholz (1966) and Steward (1972) as above). Variations in tissue N and P content (i.e., the product of nutrient concentration and plant mass) closely paralleled those of respective N ($r = 0.87$, $P = 0.0003$) and P concentration ($r = 0.75$, $P = 0.0052$), with minimal levels of both nutrients contained in fragments exposed for 4 weeks.

Although all fragments were shown to be viable under laboratory conditions, growth varied significantly among the different exposure groups (Figure 6). Fragments exposed for 1 week produced as much biomass after planting as did those that were not exposed (0 weeks in the river), while fragments exposed for 4 weeks exhibited the lowest levels of production. Maximum biomass was accrued in fragments allowed to float for 2 weeks probably due to their having a high carbohydrate content (Table 9) for establishment, in combination with roots for immediate uptake of sediment nutrients. Patterns of shoot elongation and branching were somewhat similar to biomass responses (Figure 6), most notably showing sharp reductions in height and number of apices in the 4-week exposure group.

In contrast with the above viability results, it should be noted that none of the fragments grew successfully in flats placed at the bottom of the DM (unvegetated) site. Rybicki and Carter (1995) have suggested that low light conditions may have prevented these cuttings from growing fast enough to overcome damage due to breakage, loss of leaves, and heavy epiphyte infestations.

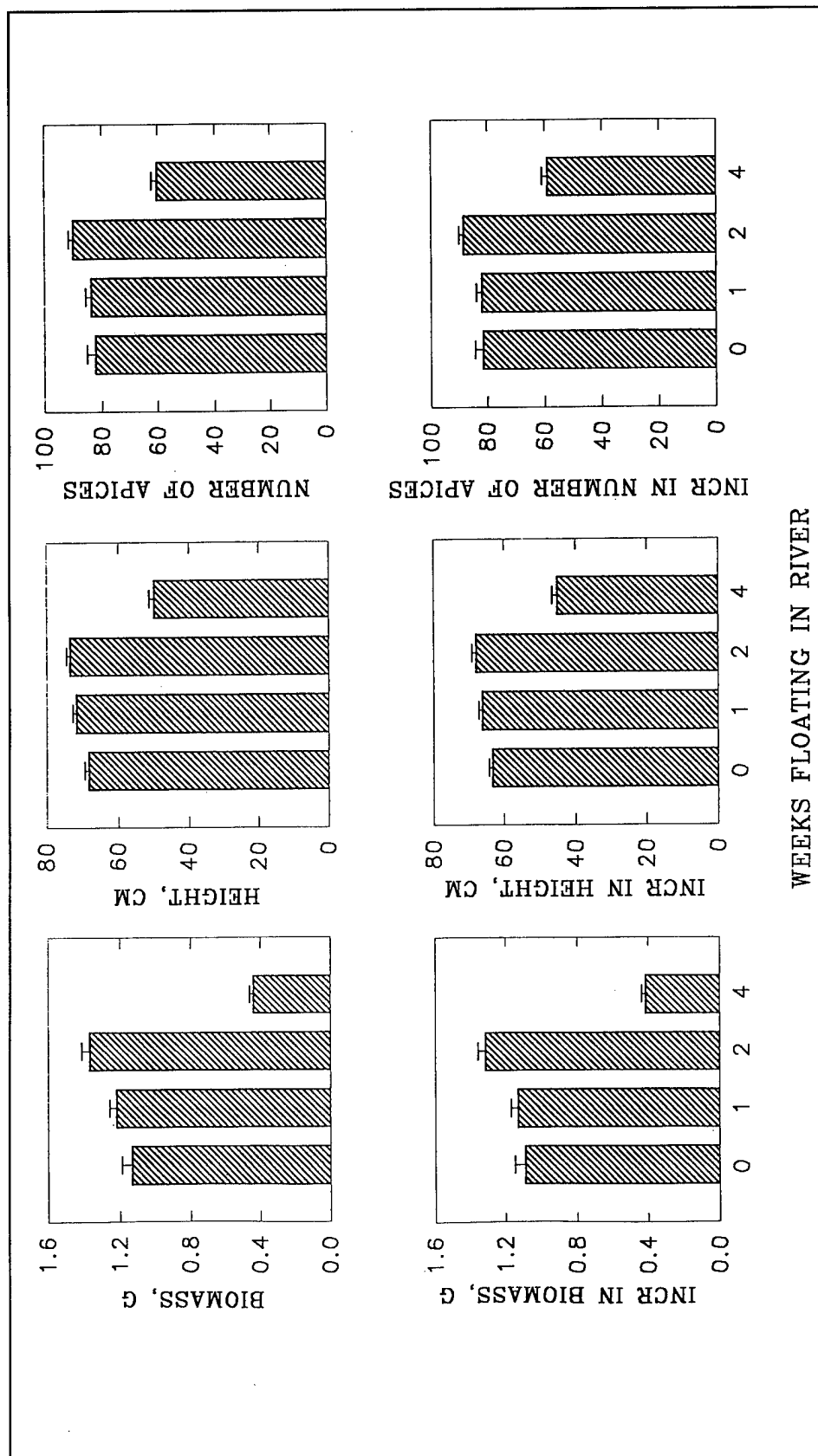


Figure 6. Growth responses of *Hydrilla* fragments after floating for different time intervals in tidal Potomac River, DM-4R site (Top panels indicate final levels achieved after 3 1/2 weeks of growth; bottom panels indicate increase (INCR) in response over initial levels. Values are means with standard errors ($n = 20$) for biomass (left), shoot height (middle), and number of apices (right))

Table 9

Percentages of Carbohydrates (i.e., free sugar (FREE), starch (STAR), and total nonstructural (TNC)) and Quantities of Carbohydrates (i.e., QFREE, QSTAR, and QTNC (mg)) in Fragments of *Hydrilla* Floated for Different Time Intervals in Tidal Potomac River¹

Weeks Floated in River	FREE	STAR	TNC	QFREE	QSTAR	QTNC
0	0.468 c	0.528 d	1.055 d	0.176 c	0.199 d	0.397 d
1	0.815 c	2.509 c	3.603 c	0.719 b	2.216 b	3.181 b
2	2.708 b	7.026 a	10.515 a	1.595 a	4.138 a	6.193 a
4	3.156 a	4.300 b	7.933 b	0.726 b	0.989 c	1.825 c

¹ Values are means based on analysis of three replicate plant samples. Within each column, means sharing the same letter do not differ at the 5-percent level of significance (Duncan's Multiple Range Test).

Study 4: Number and Viability of Transported Propagules

Onsite observations (cf. Rybicki and Carter 1995) as well as counts of fragments collected on the nets and traps indicated that submersed macrophyte propagules were indeed being transported into the unvegetated DM, HC, and PB sites. Fragments of various species caught at the DM site far exceeded those caught at the PB or the HC site. U.S. Geological Survey data indicate that seven submersed macrophyte species (*Ceratophyllum demersum* L., *Hydrilla verticillata* (L.f.) Caspary, *Myriophyllum spicatum* L., *Najas minor* All., *Vallisneria americana* Michx., *Potamogeton pectinatus* L., and *Zannichellia palustris* L.) reached sites DM and PB, while only four of the seven (*H. verticillata*, *M. spicatum*, *V. americana*, and *C. demersum*) reached the HC site. Fragments of *C. demersum* and *H. verticillata* were more abundant than all other species at sites PB and DM; at the HC site, *Hydrilla* fragments exceeded those of other submersed macrophytes.

Figure 7 shows mean fluxes (i.e., fragments/square meter/day and fresh weight/square meter/day) of *Hydrilla* collected on traps as determined (at WES) for the period 7 June to 28 September 1994. At the PB site, mean fluxes of *Hydrilla* peaked early in the growing season (22 June), but were highest in late September at the HC and DM sites. *Hydrilla* senescence in September was reflected by increased overall fluxes of fragments and fresh weight during that particular month (see synopsis; Figure 8). Fluxes on traps and nets (not shown) were usually similar but smaller on the nets, indicating fewer fragments and less biomass suspended in the water column than descending to bottom sediments.

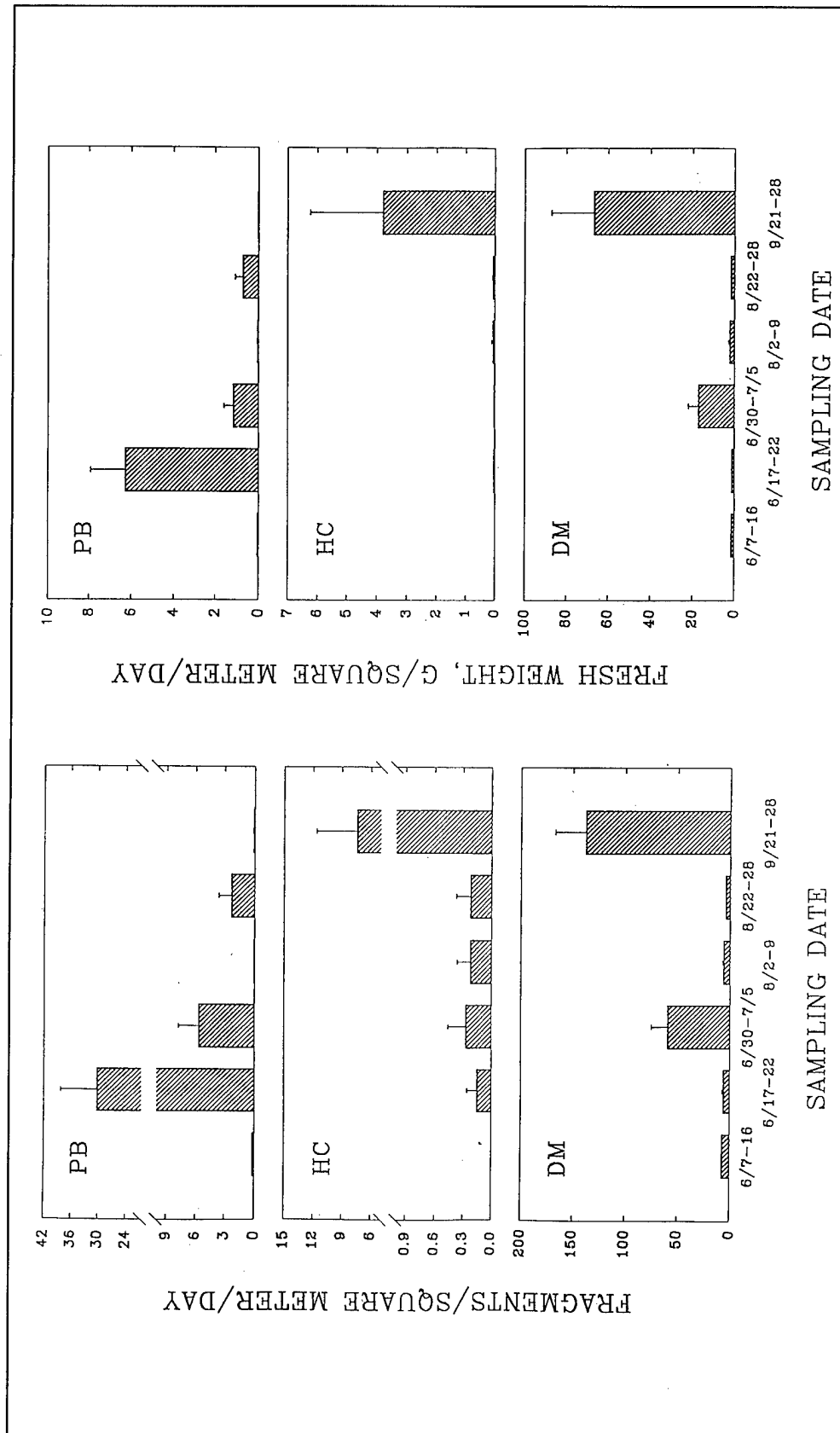


Figure 7. Mean fluxes of *Hydrilla* fragments and fresh weight deposited on traps from 7 June through 28 September 1994 in tidal Potomac River at three unvegetated sites (PB, DM, and HC) (Values are means with standard errors ($n = 12$))

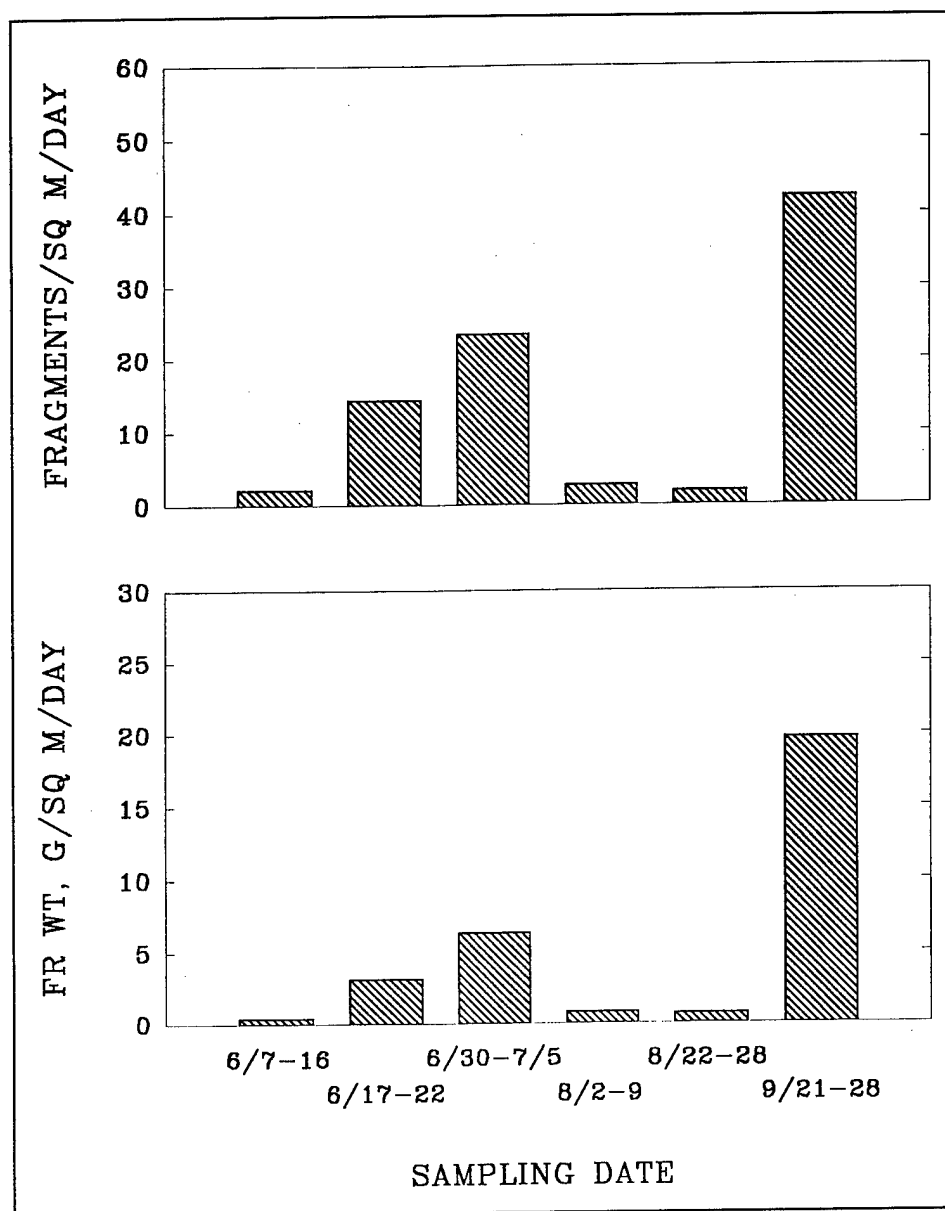


Figure 8. Synopsis of flux of *Hydrilla* fragments and fresh weight, from 7 June through 28 September 1994, for all unvegetated sites and apparatuses (traps and nets) combined

Laboratory tests showed that the viability of *Hydrilla* fragments peaked at all three sites in early June (Table 10), generally coinciding with the period of rapid increases in the length and biomass of *Hydrilla* plants in the field.¹ Over the remainder of the summer, the viability of fragments gradually decreased, reaching estimated minima of 61, 49, and 20 percent at DM, HC,

¹ Personal Communication, 1995, Nancy Rybicki, Hydrologist, U.S. Geological Survey, Reston, VA.

Table 10 Percent Viability of <i>Hydrilla</i> Fragments Over 1994 Growing Season From DM-4R (DM), Hunting Creek (HC), and Pohick Bay (PB) Sites									
Date	DM			HC			PB		
	<i>n</i> ¹	No. Viable	%	<i>n</i>	No. Viable	%	<i>n</i>	No. Viable	%
6/7-16	85	73	86	2	2	100	11	11	100
6/17-22	125	106	85	16	12	75	125	114	91
6/30-7/5	124	76	61	25	20	80	49	33	67
8/2-9	84	47	56	9	8	89	14	10	71
8/22-28	29	21	72	11	7	64	18	12	67
9/21-28	125	76	61	68	33	49	10	2	20
All Dates	572	399	70	131	82	63	227	182	80
¹ <i>n</i> = the number of fragments tested from traps and nets combined.									

and PB, respectively, by early fall (21-28 September). Morphological determinations made during viability testing indicated mortality to be highest among fragments that had sustained the most injury. These fragments were typically short (< 10 cm in length) and brittle, with few if any intact roots, leaves or branches.

4 General Discussion and Conclusions

Results of these studies collectively provide substantial evidence indicating that over the growing season of 1994 viable propagules of *Hydrilla* and other submersed macrophytes (mostly fragments) were transported into unvegetated Potomac River sites. However, several factors appear to have hindered the ability of these propagules to establish new colonies. In general, submersed angiosperms such as *Hydrilla* are deficient in mechanical tissues. Thus, fragments of these macrophytes are highly susceptible to damage imposed by strong currents and wave action (cf. Steward 1992). In the studies for this report, fragment damage incurred during prolonged periods of flotation appeared to increase the risk of mortality due to a loss of structure (e.g., roots, leaves, and apices) and physical deterioration. Rybicki and Carter (1995) have suggested that poor water clarity may have diminished fragment survival due to shading by suspended solids and phytoplankton populations. An additional possibility is that sediment nutrient levels were growth limiting; bioassay results obtained here revealed marked reductions in the growth of *Hydrilla* on HC and DM sediments due to negative influences on fragment nutrition (see below).

Although field populations of tubers and turions of *Hydrilla* have not been widely examined, the mass and viability of these propagules appear to vary considerably (this study and more so in Spencer et al. (1987)). In some species, e.g., *Vallisneria americana* Michx., mortality tends to be higher for smaller than for larger tubers (Titus and Hoover 1991; Rybicki and Carter 1995). In *Potamogeton pectinatus* L., successful emergence from sediment, initial growth rate, and resistance to adverse conditions, e.g., herbicide treatment, were found to be positively related to tuber fresh weight (Spencer 1986, 1987; Spencer, Ksander, and Whitehand 1989). Differences in propagule mass affected by changes in environmental conditions may also influence these processes in *Hydrilla*. A greater understanding of the mass-related vigor of *Hydrilla* tubers and turions would be useful in predicting recruitment and postgermination success of this species in the field.

Findings of this report substantiate the proposal of Titus and Hoover (1991) that propagules containing larger quantities of carbohydrates are more likely to support the development of a root system and vertical growth in the

water column to where net photosynthesis can occur. This proposal is based in part on studies showing that low concentrations of total nonstructural carbohydrates increased mortality in fragments of *Myriophyllum spicatum* L. (Kimbél 1982). In *Hydrilla* fragments examined in the present studies, increases in carbohydrate levels and concomitant root development (observed over the initial 2 weeks of flotation) provided immediate advantages for propagule establishment and uptake of sediment nutrients. The early establishment of the fragments enabled a longer time for growth, resulting in greater morphological development (i.e., plant height and branches) and biomass production.

Increases in biomass and branch development in *Hydrilla* fragments while floating in the river ultimately resulted in considerable N dilution in fragment tissues, suggesting a dependence on internal N levels to support modifications in fragment structure. These changes in fragment structure provided greater surface area for photosynthesis and may have contributed to observed increases in internal carbohydrate content. Although nutritional data are not available, similar structural changes have been reported for fragments of *M. spicatum* as well. Laboratory studies by Madsen, Eichler, and Boylen (1988) showed that fragments of this species increased significantly in length and biomass during 36 days of incubation in oligotrophic waters of Lake George. Under field conditions, the survival of fragments may be enhanced by structural modifications that augment internal carbohydrate pools (cf. Kimbél 1982; Titus and Hoover 1991). Considering the importance of fragments to the colonization of submersed macrophytes (Sculthorpe 1967), mechanisms affecting the vigor of these propagules need to be better understood.

In the sediment bioassay, low N availability inhibited growth of *Hydrilla* fragments on sediments from sites HC and DM, suggesting the potential for N-limited growth on these sediments in the field. It should be noted, however, that this bioassay examined growth on sediments in a closed system. Therefore, the results presented here provide only an indication of possible sediment effects in the field with little or no outside nutrient inputs. To assess more effectively the potential for nutrient (N) limitation requires information on nutrient replenishment rates (cf. Barko, Adams, and Clesceri 1986; Barko et al. 1988; James and Barko 1990). Under natural conditions, numerous biological and limnological processes (e.g., sedimentation, mineralization, bioturbation, diffusion, erosion, and plant uptake) can contribute to changes in the availability of N and other nutrients in sediment (Barko, Gunnison, and Carpenter 1991). These processes may be important in affecting propagule survival on sediments at these Potomac River sites.

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13. ABSTRACT (Maximum 200 words) <p>The availability of viable propagules of <i>Hydrilla verticillata</i> (L.f.) Caspary in the tidal Potomac River was examined in a series of studies conducted in 1994. The studies were performed over time frames selected to coincide with the seasonal production of different propagule types. Five sites were chosen based on observations made during the previous (1993) growing season, indicating that two of the sites were vegetated—the Greenway Flats (GF) site supporting <i>Myriophyllum spicatum</i> L. and <i>Hydrilla</i>, and the Dempsey Dumpster (DD) site, <i>Vallisneria americana</i> Michx.—while the three remaining sites, DM-4R (DM), Pohick Bay (PB), and Hunting Creek (HC), were unvegetated.</p> <p>All five sites were sampled in March for overwintered tubers and turions. Nearly all of the propagules collected (99 percent, $n = 131$) were found at the GF site. Maximum propagule densities (66 tubers and 157 turions m^{-2}) at this location occurred between 1.0- and 1.5-m sampling depths. Under laboratory conditions, germination was 78 percent for tubers ($n = 49$) and 60 percent for turions ($n = 81$) and was closely related to propagule fresh weight.</p> <p>Measurements of propagule transport from May through September confirmed that submersed macrophyte propagules (predominately <i>Hydrilla</i> fragments) were reaching the three unvegetated sites. Mean rates of transport</p> <p style="text-align: right;">(Continued)</p>				
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for *Hydrilla* were 1.3, 5.6, and 37 fragments $\text{m}^{-2} \text{day}^{-1}$, with total fresh weights of 0.58, 1.21, and 13.9 g $\text{m}^{-2} \text{day}^{-1}$ at sites HC, PB, and DM, respectively. A strong seasonal trend was noted in the transport of *Hydrilla* fragments, with peak numbers and fresh weights occurring during senescence in late September.

In *Hydrilla* fragments tested in the laboratory from the three unvegetated sites, the overall viability for the season was approximately 70 percent ($n = 641$). Viability peaked at 90 percent ($n = 98$) in early June, generally coinciding with the period of rapid increases in length and biomass of *Hydrilla* plants in the field. Over the remainder of the summer, viability percentages gradually declined, reaching a minimum of 55 percent ($n = 203$) by early fall.

Laboratory studies of *Hydrilla* fragments (apical stems) left floating in the river for 0, 1, 2, and 4 weeks showed that all fragments were viable after each treatment. Increases in biomass after planting were greatest in fragments that had floated for 2 weeks, probably due to their having the highest carbohydrate levels and roots for immediate uptake of sediment nutrients. Fragments that had floated for 4 weeks were in relatively poor condition due to breakage and other damage and consequently produced the least biomass after planting.

Viability studies revealed that live fragments of *Hydrilla* were being deposited at the unvegetated sites; however, these propagules were largely unsuccessful in establishing new colonies. Possible limiting factors are considered based on collective experimental results, including potential effects of nitrogen and phosphorus in sediments limiting propagule growth.

14. (Concluded).

Biomass production

Fragmentation

Growth

Hydrilla

Mortality

Nutrient content

Potomac River

Propagules

Reproduction

Survival

Tubers

Turions

Viability